[Document Name] Description

[Title of the Invention] Infection defense functional reinforcement agent

[Claim(s)]

[Claim 1] The infection defense functional reinforcement agent characterized by containing 3000 or less molecular weight [which is obtained by protease decomposing the protein in royal jelly] peptide as an active ingredient.

[Claim 2] The infection defense functional reinforcement agent according to claim 1 obtained by protease decomposing raw royal jelly directly.

[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the infection defense functional reinforcement agent which makes an active ingredient the peptide obtained by protease decomposing the

protein in royal jelly.

[0002]

[Description of the Prior Art] Royal jelly is a substance with the strong acidity which ****ed the milk white secreted from a young worker bee's secretion line (a bottom pharynx line, large ****), and serves as special food for breeding a queen bee. About the ingredient of royal jelly, although there is also still an unknown point, nutritional information, such as vitamin, a mineral, amino acid, acetylcholine, 10-hydroxy decene acid, sterol, and hormone, is included with sufficient balance, and having desirable physiology activity to a human body is known. Therefore, it is used for many years as health food, such as a raw type, a capsule type, and a drink type, medical supplies, cosmetics, etc.

[0003] Various reports are made about the physiological function of royal jelly until now. For example, IgG of royal jelly [in / by Murakami and others / the system of in vitro], IgM The production promotion operation (Agric.Biol.Chem., 54 (4), 1087-1089, 1990) and the production promotion operation ('90 and '91 collection of agricultural chemistry society lecture summaries) of IFN-gamma are reported. Moreover, the multiplication facilitatory effect (92' the collection of biochemistry meeting lecture summaries and No.2035) of the human single ball cultured cell is reported by Yonekura and others.

[0004] Moreover, this invention persons are in vivo of royal jelly. When the influence which it has on a physiological function was considered and the oral ingestion of royal jelly defended infection of an E. coli bacillus or a salmonella, the effective thing ('93 collection of agricultural chemistry society lecture summaries) was found out. Moreover, the activity reported the strong thing ('94 collection of agricultural chemistry society lecture summaries) in a part for 3000 or less molecular weight [of royal jelly] low-molecular drawing.

[0005] On the other hand, by processing raw royal jelly by two or more kinds of protease from which the operation part to a ground substance differs, this invention persons contain the same ingredient as raw royal jelly, and, moreover, are transparent. It found out that the transparent royal jelly solution which is excellent in stability, and is easy to add to a drink etc., therefore is

easy to carry out oral ingestion was obtained, and has already applied as Tokuganhei3-255921.

[0006]

[Problem to be solved by the invention] Although royal jelly has an infection defense functional improvement operation as mentioned above, raw royal jelly [consistency] It is hard to melt into water, when peculiar flavor which is fishy is had and eaten in a yellowish-white color, there is an intense stimulus with which a throat is stabbed, and it is still more unstable to processing of heating etc., and has the problem of being hard to save in normal temperature.

[0007] Therefore, in order to utilize effectively an infection defense functional improvement operation of royal jelly, when it was hard to add even if it was going to add for food etc., and it added to the drink, muddiness was produced, and there was a problem of being hard to eat for the flavor and stimulus, further.

[0008] Moreover, although especially the infection defense functional improvement operation was strong for the 3000 or less molecular weight [of royal jelly] low-molecular ingredient, there was a problem that there was little quantity of a low-molecular ingredient and expensive royal jelly could not be used effectively, in view of the whole royal jelly.

[0009] This invention was made in view of the above-mentioned problem, the purpose can employ effectively an infection defense functional improvement operation of royal jelly efficiently, and tends to add it to food, a drink, etc., and it is in offering the infection defense functional reinforcement agent which is easy to carry out oral ingestion.

[0010]

[Means for solving problem] 3000 or less molecular weight [which disassembled and obtained the protein in royal jelly by protease] peptide finds out having the outstanding infection

defense functional enhancement effect, and this invention persons came to complete this invention based on this fact, as a result of inquiring wholeheartedly, in order to attain the above-mentioned purpose.

[0011] That is, the infection defense functional reinforcement agent of this invention is characterized by containing 3000 or less molecular weight [which is obtained by protease decomposing the protein in royal jelly] peptide as an active ingredient.

[0012] Hereafter, a desirable mode is mentioned and this invention is explained in detail. The infection defense functional reinforcement agent of this invention should just contain 3000 or less molecular weight [which disassembled and obtained the protein in royal jelly by protease] peptide. [this peptide / the crude protein solution separated from the water suspension liquid of raw royal jelly, or raw royal jelly by curing salting etc.] Protease and two or more kinds of protease from which the operation part to a ground substance differs preferably are obtained simultaneous or by carrying out consecutive addition, and holding and carrying out an enzyme reaction to the temperature beyond room temperature.

[0013] As protease, in this case, the acid protease of a microbe or the vegetable origin, As for digestive enzymes of mammal origin, such as neutral protease, alkaline protease, and pepsin, pan-creatine, etc., it is desirable to be usually able to use what is used for food processing, and to choose and use two or more kinds from which the operation part to a ground substance differs out of these.

[0014] As for the processing by protease, it is desirable to close, when a proteinic decomposition rate is measured temporally and a decomposition rate reaches the highest, and although the last decomposition rate changes with protease to be used, it is desirable that a decomposition rate carries out 75% or more until it becomes 85% or more preferably. In addition, the details of the above-mentioned manufacturing process are indicated to JP,H3-255921,A.

[0015] In this way, the decomposed materials by the protease of the obtained royal jelly contain 3000 or less-molecular weight peptide. Moreover, when protease is made to act on the

water suspension liquid of raw royal jelly directly, protein is disassembled and the same ingredient as raw royal jelly is contained except having peptide-ized.

[0016] When it is made solution, it is transparent, and it excels in stability, therefore is easy to add to a drink etc., and easy to carry out oral ingestion of the decomposed materials of this royal jelly. In addition, the decomposed materials of this royal jelly may be used by the shape of solution as it is, by freeze-drying, spray dry, or other means, may be powder-ized and may be used.

[0017] The infection defense functional reinforcement agent of this invention is useful to infection defense of pathogenic bacteria or a virus, and effective for especially bacterial infection defense. In addition, taking in from every day is desirable as health food or medical supplies rather than takes in in medical treatment after being infected since it has the preclusive operation of being hard to be infected with bacteria or a virus, and being easy to cure even if infected when it takes in beforehand.

[0018] As health food or medical supplies, the infection defense functional reinforcement agent of this invention may be made into a concentrate, a tablet, a capsule agent, etc., and oral ingestion may be carried out as it is, for example, it may be mixed and taken in to honey etc., and may be mixed and taken in to a nutrition supplement drink, soft drinks, etc. In addition, intake is raw royal jelly conversion and it is desirable to consider it per day and as about 0.3 - 6g.

[0019]

[Function] After carrying out oral ingestion of the infection defense functional reinforcement agent of this invention to a mouse, when the E. coli bacillus was made to carry out forcible infection, as shown in the work example mentioned later, it turned out that the outstanding infection defense functional enhancing effect is brought about. Therefore, also when man is made to take in, it is thought that the same infection defense functional enhancing effect is acquired.

[0020] Moreover, [when oral ingestion of the royal jelly is carried out as it was, the protein of royal jelly is hard to disassemble in a digestive enzyme in the living body, and digestion and absorption are also hard to be carried out, but] Since the infection defense functional reinforcement agent of this invention makes an active ingredient the peptide which obtained the protein in royal jelly by having carried out enzyme decomposition beforehand, in a body, it digests, and is easy to be absorbed, and it is thought that the above-mentioned infection defense functional enhancing effect is heightened.

[0021] Furthermore, since most stimuli with which it becomes easy to melt into water and characteristic acridity and a throat are stabbed will be lost if royal jelly is processed by protease, it is easy to add to food, a drink, etc., and becomes easy to carry out oral ingestion.

[0022] When the infection defense functional reinforcement agent of this invention is obtained by disassembling raw royal jelly by protease directly Since protein is disassembled and the same ingredient as raw royal jelly is contained except having peptide-ized, nourishment tonic property, various kinds of other physiology activity effects, etc. of the royal jelly known conventionally are expectable.

[0023]

[Working example] Warm water is added to work-example 1 student royal jelly 100 g, water suspension liquid 1 kg of royal jelly is prepared, sodium hydroxide solution is used 20%, and it is pH 4 It carried out. To this soil suspension, pepsin (1:10,000, sigma company make) 0.1 g is added, and it is 4 at 45 degrees C. Time enzyme processing was performed and pepsin processing liquid was obtained.

[0024] Next, sodium hydroxide solution is used for this pepsin processing liquid 20%, and it is pH 8 After adjusting, trypsin (potency 2,000,000 unit/g, sigma company make) 1 g is added, and it is 4 at 45 degrees C. Time enzyme processing was performed.

[0025] After ending enzyme processing, citrate solution is used 10%, and it is pH 5.5 It adjusted, at 80 degrees C, it heats for 10 minutes and enzyme was deactivated, subsequently, it filtered, non-decomposed protein and an insoluble residual substance were removed, and transparent solution was obtained.

[0026] Subsequently, the obtained solution was freeze-dried and the protease processing powder of royal jelly was obtained.

[0027] In work-example 2 work example 1, it is 16 hours about pepsin processing time, Carrying out trypsin processing time in 16 hours, other conditions obtained the protease processing powder of royal jelly like the work example 1.

[0028] To work-example 3 student royal jelly 1 kg, the 0.1 M phosphate buffer solution (pH 7.0) was added, and it was considered as royal jelly solution 10% at it. It added and curing salting of the ammonium sulfate was carried out to this solution so that it might become saturation 60%. A part for this precipitation drawing was dialyzed, subsequently it freeze-dried, and the powder which consists of a part for the protein drawing of royal jelly was obtained.

[0029] 50g of obtained powder After adding water and being referred to as 2 L (liter), 6 N chloride is used, and it is pH 4.0 It adjusted. In this solution, pepsin (1:10,000, sigma company make) 0.1 g is added, and it is 6 at 37 degrees C. Time enzyme processing was performed and pepsin processing liquid was obtained.

[0030] Next, sodium hydroxide solution is used for this pepsin processing liquid 20%, and it is pH 8 After adjusting, trypsin (potency 2,000,000 unit/g, sigma company make) 0.1 g was added, and enzyme processing was performed at 40 degrees C for 16 hours.

[0031] After ending enzyme processing, citrate solution is used 10%, and it is pH 5.5 It adjusted, at 80 degrees C, it heats for 10 minutes and enzyme was deactivated, subsequently, it filtered, the foreign substance was removed, and transparent solution was obtained.

[0032] Subsequently, the obtained solution was freeze-dried and the protease processing powder for protein drawing of royal jelly was obtained.

[0033] Comparative example 1 student royal jelly 100 g It is volume and water was added in equivalent amount, it freeze-dried and royal jelly powder was obtained.

[0034] Enzyme processing was not carried out but powder which consists of a part for the protein drawing of the royal jelly manufactured by curing salting in comparative example 2 work example 3 was used as the powder of a comparative example 2.

[0035] About the powder of example of experiment 1 (measurement of a proteinic decomposition rate) work examples 1-3, the proteinic decomposition rate was measured by the method shown below.

[0036] After having added an equivalent amount of 10% trichloroacetic acid (TCA) to sample 2.0 ml, performing centrifugal separation and diluting top [this] **, a **** fixed quantity was carried out by the Raleigh method, and transition of the quantity of a TCA soluble ingredient was investigated. In addition, using the solution which added an equivalent amount of water, a **** fixed quantity of the crude protein in royal jelly was similarly carried out instead of TCA solution, and it was asked for it. And it asked for the proteinic decomposition rate by the formula shown in the one following.

[0037]

[Mathematical formula 1] Decomposition rate ={(c-b)/(a-b)} x100%a: Content of the TCA soluble ingredient of the content c:enzyme processing sample of the TCA soluble ingredient of the crude protein content b:royal jelly solution of royal jelly solution

[0038] The work example 2 was [the work example 3 of the powder of the work example 1 of

the decomposition rate of the protein for which it asked by the above-mentioned method] 98% 96% 85%.

[0039] Molecular weight distribution was measured and compared with the protease processing powder of the royal jelly obtained in the example of experiment 2 (measurement of molecular weight distribution) work example 2 about raw royal jelly. In addition, high speed liquid chromatography performed the measuring method. The result is shown in drawing 1. In drawing 1, a solid line B shows the result of raw royal jelly as a result of the protease processing powder of the royal jelly with which the dashed line A was obtained in the work example 2.

[0040] It turns out that a 10,000 or more-molecular weight thing is almost lost to the powder obtained in the work example 2, 3000 or less-molecular weight peptide has become it from the result of drawing 1 with the main ingredients, and many things with 100-1000 molecular weights remain especially. This was checked also from results, such as electrophoresis.

[0041] The ingredient was analyzed about the protease processing powder of the royal jelly obtained in the example of experiment 3 (component analysis) work example 2, and the raw royal jelly powder obtained by the comparative example 1, and the composition was compared. The result is shown in Table 1.

[0042]

[Table 1] ** ----- ** --------- ** --------- ** ** Powder [of the powder ** comparative example 1 of the ** work example 2] ** ** ----- ** --------- ** --------- ** ** crude protein ** 36.0 ** 37.5 ** ** non-fibrous carbohydrates ** 47.0 ** 45.0 ** ** lipid ** 7.5 ** 7.8 ** ** decene acid ** 6.0 ** 6.2 ** ** moisture ** 3.0 ** 2.5 ** **-----**---------**---------** (the inside of Table 1 and a unit are weight %, and are =) Decene acid means 10-hydroxy delta-decene acid.

[0043] The result of Table 1 shows that the composition is almost the same as the protease processing powder of the royal jelly obtained in the work example 2, and the raw royal jelly powder obtained by the comparative example 1. That is, it turns out that the ingredient as raw royal jelly with protease processing powder of the royal jelly obtained in the work example 2 same except protein having been disassembled and having peptide-ized is contained.

[0044] In addition, even if the powder obtained in the work examples 1-3 dissolved easily and it ate it as it was, without carrying out coagulation sedimentation like raw royal jelly even if it added to the weak acidic drink, it was what does not have a stimulus with which a throat is stabbed, either and has some taste and sweetness rather.

[0045] The powder of example of experiment 4 work examples 1 and 2 and a comparative example 1 was administered orally to the mouse (ICR mouse (crj:CD-1) and 5 week-old SPF) of the male of ten per group by the sonde at a rate of 2 g/kg/mousu. In addition, the contrast division was medicated only with the phosphorus acid buffering physiological saline.

[0046] The intravenous injection of the E. coli bacillus liquid (E. coll.KC-14) 4 x107CFU/0.2 ml/mouse of the minimum fatality given dose was given after 24-hour progress. 4 After the day, it asked for the probability of survival with the number of the mice which survive. The result is shown in drawing 2.

[0047] Although the result of drawing 2 to the probability of survival was 0 % in the contrast division, it was 60% 40% in the royal jelly medication division of the comparative example 1 in

both the protease processing thing medication divisions of the royal jelly of a work example 1 and a work example 2. Thus, when there is an infection defense functional enhancing effect in royal jelly and it is decomposed by protease, it turns out that the effect increases remarkably.

[0048] The defense effect as opposed to [changed the example of experiment 5 internal-use sample to the powder of the work example 3 and the comparative example 2, and also] coliform bacillus infection of a mouse like the example 4 of an experiment was investigated. The result is shown in drawing 3.

[0049] The result of drawing 3 to the probability of survival was [in the contrast division] 80% 50% in the protease processing thing medication division for royal jelly protein drawing of the work example 3 in 0 % and the royal jelly protein drawing part medication division of the comparative example 2. Thus, it turns out that the active ingredient of the infection defense functional enhancing effect of royal jelly is in a part for protein drawing, and activity increases further by processing it by protease.

[0050]

[Effect of the Invention] As explained above, the infection defense functional reinforcement agent of this invention contains 3000 or less molecular weight [which is obtained by protease decomposing the protein in royal jelly] peptide as an active ingredient, and an infection defense functional enhancing effect is expected by carrying out oral ingestion of this.

[0051] Moreover, since most stimuli with which it becomes easy to melt into water and characteristic acridity and a throat are stabbed will be lost if royal jelly is processed by protease, it is easy to add to food, a drink, etc., and becomes easy to carry out oral ingestion.

[0052] Furthermore, in what obtained raw royal jelly by having decomposed by protease directly, protein is disassembled, and since the same ingredient as raw royal jelly is contained except having peptide-ized, not only an infection defense functional enhancing effect but other effects of nourishment strong ** of royal jelly are expectable.

[Brief Description of the Drawings]

[Drawing 1] It is the chart showing the molecular weight distribution of raw royal jelly with the protease processing powder of the royal jelly obtained in the work example 2.

[Drawing 2] It is the chart showing the probability of survival at the time of carrying out coliform bacillus infection of the mouse which administered orally the powder of work examples 1 and 2 and a comparative example 1.

[Drawing 3] It is the chart showing the probability of survival at the time of carrying out coliform bacillus infection of the mouse which administered orally the powder of the work example 3 and the comparative example 2.

[Translation done.]